Investigating the antibacterial properties of the *Salix matsudana*

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Abstract

Plants in the genus of Salix were long believed to have medicinal properties. It contains salicin, which acts similarly to and is used to produce acetylsalicylic acid, also known as Aspirin. Aspirin is used to treat pain, fever and inflammation as well as heart attacks, and is also known to lower cancer risks. Plants in the genus of Salix are also widely known to have antibacterial properties. Therefore, this study aims to investigate the antibacterial properties of the *Salix matsudana*. Leaves and bark powder were soaked in ethanol for 6 days. The surface of an MHA plate with agar was coated with *Escherichia coli.* After the solutions of the leaf and bark extract, as well as 10% bleach for the positive control and 50% ethanol for the negative control, were pipetted into holes on the MHA plates, they were incubated and their zones of inhibition were measured and recorded. Both the solutions had zones of inhibition larger than the negative control, proving antibacterial activity, but not more than the positive control. With its antibacterial properties in mind, Salix matsudana could be used for food preservatives, soaps or medicinal drugs.

1. Introduction

The *Salix matsudana* is also known as the Chinese willow. It is a species of willow native to northeastern China. The *Salix matsudana* trees were planted in Hwa Chong Institution after Deputy Principal Mr Tan Pheng Tiong wanted a scenic effect in the school, and also because the weeping willow, also known as *Salix babylonica*, was unable to grow well under Singapore's hot climate. The willow has long been able to receive attention for its medical properties. This is because the willow contains salicin, which acts similarly to and which, through oxidation and hydrolysis, forms salicylic acid, and eventually led to the discovery of Aspirin (Mahdi et al., 2006). Aspirin can inhibit the spread of cancer, in addition to being used to treat pain and inflammation. For example, generally, aspirin induces a dose-dependent reduction in the proliferation rate of HT-29 cells (Shiff et al., 1996). The sodium salt of aspirin also significantly inhibits cellular proliferation of human pancreatic

cancer cell lines in a dose-dependent manner (Perugini et al., 2000). Similar effects of aspirin on the growth of three different human colon cancer cell populations (HCT116, HCT116 + chr3 and SW480) but at different rates were also found. (Goel et al., 2003). Plants in the genus of *Salix* were also long believed to have antibacterial properties. For example, after researching them through research articles, it was found that the *Salix subserrata*, *Salix alba* and *Salix babylonica* had promising antibacterial results. Four compounds out of eight in total found in the bark and leaves of Salix subserrata showed good antibacterial and algicidal and moderate antifungal properties (Hussain et al., 2011). It was found that the number of protozoa in the rumen of lambs decreased significantly after two months of oral administration of extract from the leaves of S. babylonica in an amount of 30 ml per day (Hernandez et al., 2014). There was also good antimycotic activity of 20% ethanolic extract of S. babylonica against Fusarium oxysporum. (Sati et al., 2013). This study aimed to find out if the *Salix matsudana* in Hwa Chong Institution was able to demonstrate antibacterial properties like many of the other plants in the genus of *Salix.* These findings would be useful because antibacterial items are used frequently in day to day life, such as for food preservatives, medicine or soap. With such medicinal value, it would be extremely beneficial for *Salix matsudana* to also have antibacterial activity as well, as an added bonus to its place in the pharmaceutical industry.

2. Objectives and hypotheses

2.1 Objectives

The objective of this study is to test the antibacterial properties of *Salix matsudana* leaves and bark on *Escherichia coli*, and possibly other microorganisms.

2.2 Hypothesis

The hypothesis of this study is that *Salix matsudana's* leaves and bark will have a significant antibacterial effect on *Escherichia coli.*

3. Methods and Materials

3.1 Materials

2 grams of *Salix matsudana* leaf powder and 2 grams of *Salix matsudana* bark powder were used. The leaves and bark were collected from the *Salix matsudana* trees by the Clock Tower. 3 Petri dishes with MHA agar, samples of *Escherichia coli,* 10% bleach solution, ethanol, a grinder, a pipette and an incubator were obtained from the SRC.

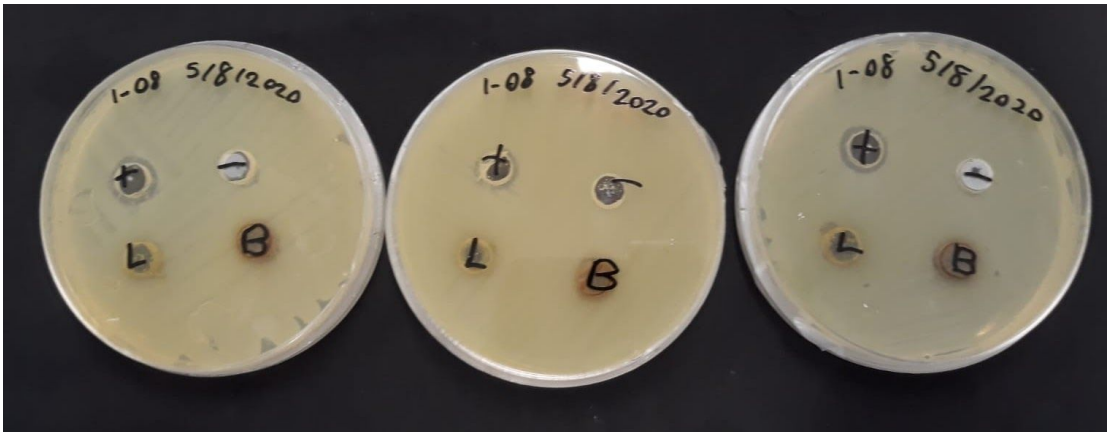
3.2 Methodology

After leaves and bark from the *Salix matsudana* tree were left to dry for 3 days, the leaves and bark were crushed into a powder using a grinder. 2g of the leaf powder was soaked in 15ml of ethanol. 2g of the bark powder was also soaked in 15g of ethanol. The solutions were left for another 6 days. MHA plates were prepared with 20ml of agar and the agar were allowed to cool and solidify overnight. To prepare the agar, the agar powder was mixed in water. 3 MHA plates were prepared for there to be triplicates to ensure accuracy. A cotton swab was heated up by the fire in the laminar fume hood. The cotton swab inoculated with E. coli. was used to spread the bacteria onto each MHA plate. Four 7mm holes were poked in each of the three MHA plates. In a clockwise direction, the positive control, negative control, bark solution and leaf solution were pipetted into the holes on each of the MHA plates after being heated over the flame. 10% bleach was used as the positive control and 50% ethanol was used as the negative control. All 3 MHA plates were incubated at 37 degrees overnight. The zone of inhibition for each solution was read using a ruler at the lab over a black piece of cloth.

4. Results and Discussion

4.1 Results

Fig. 1: a picture of the results of the experiment.



Results show that leaf and bark extracts had antibacterial effects but less efficacy than the positive control. As for the bark extract, there was more clearance than the leaf extract, and hence greater antibacterial effect. The bark extract had an average zone of inhibition of around 8mm, which is greater than the zone of inhibition for the leaf, which also had an average zone of inhibition of around 8mm, but worse results overall. Both extracts had a smaller zone of inhibition than the positive control, 10% bleach, which had an average zone of inhibition of about 13cm.

Table 1: the zones of inhibition for each MHA plate and solution used. The diameter of the hole was included in the reading, which was measured to be about 7mm. All results are ±1mm.

Zone of inhibition	Positive control 10% bleach (mm)	Negative control 50% ethanol (mm)	Bark extract (mm)	Leaf extract (mm)
1st MHA plate	12	7	8	8
2nd MHA plate	13	7	8	7
3rd MHA plate	13	7	9	8
Average	13	7	8	8

4.2 Discussion

These results show that the *Salix Matsudana* likely has the same antibacterial properties as the other plants in the genus of *Salix*, because the results are higher than that of the negative control of 50% ethanol, which is 7mm on average. The results, however, also show that there was not a great zone of inhibition, but rather only a small one. The zone of inhibition was only slightly greater than that of the 50% ethanol, enough to say that there was antibacterial property, but incomparable with that of 10% bleach. This corresponds with other studies that have also shown that plants in the genus of *Salix* all possess such antibacterial properties. Hence, it can be concluded that *Salix matsudana* plants, similarly to most other plants in the genus of *Salix*, would have antibacterial activity in their leaves and bark.

5. Conclusion and Recommendations for future work

5.1 Conclusion

Salix matsudana has antibacterial properties against *Escherichia coli.* The bark has greater antibacterial properties than the leaf of the *Salix matsudana*. They are less effective than the positive control of 10% bleach, as the leaf extract has an average zone of inhibition of approximately 8mm and the bark extract has an average zone of inhibition of approximately 8 mm. 50% ethanol, which the powders were soaked in, has negative results and does not have a zone of inhibition against *Escherichia coli.*

5.2 Recommendations for future work

Due to time constraints and lab restrictions, many of the plans could not be carried out. Here is what could have been done given the time, which may be carried out in the future.

The dilution of the extract could be changed for future work. The extract in the experiment was diluted with 5 parts of water. It could be more effective if the dilution had been changed. For example, it could be changed to be not diluted at all, or diluted with 10 parts of water. Using a larger variety of bacteria is also recommended. It is a possibility that the willow is effective against *E. coli.* but not effective against many other forms of bacteria, or even more effective against them. The willow may also be antifungal rather than antibacterial. Due to time constraints, the willow's effectiveness against fungi has not been tested. Also, a different solvent could be used. The only solvent for the experiment used for the experiment was ethanol. Other solvents, such as water, benzene or chloroform could have been used if there were no time constraints. Furthermore, the number of days the leaves and bark were soaked could have been changed. The leaves and bark were in ethanol for 6 days, and that may have been too long or too short, therefore causing the *Salix matsudana* to lose some of its potential effectiveness. Further tests can be done to find out if the *Salix matsudana* extract is edible and if it has micronutrients which can allow for it to be used as a food preservative. The willow, as said in the introduction, was also said to have medicinal value as it contained aspirin. Further tests could also be conducted to see if this is true in the *Salix matsudana* as well, and not just other types of willow. These can also help to check the amount of aspirin there is in a willow leaf or bark. If these have positive results, it can be concluded that the *Salix matsudana* has high potential for being used in the medicinal industry.

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